Orbital bleeding in rats while under diethylether anaesthesia does not influence telemetrically determined heart rate, body temperature, locomotor and eating activity when compared with anaesthesia alone

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Orbital bleeding in rats while under diethylether anaesthesia does not influence telemetrically determined heart rate, body temperature, locomotor and eating activity when compared with anaesthesia alone

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Summary

The question addressed was whether orbital bleeding in rats, while under diethylether anaesthesia, affects their locomotor activity, body core temperature, heart rate rhythm and eating pattern. Roman High Avoidance (RHA) and Roman Low Avoidance (RLA) rats were used to enhance generalization of the results. Orbital bleeding when the rats were under diethylether anaesthesia was compared with diethylether anaesthesia alone. To take into account any effects of handling, the rats were also subjected to sham anaesthesia. The RHA rats urinated more during anaesthesia, needed more time to recover from the anaesthesia and showed a greater endocrine stress response to diethylether anaesthesia when compared with the RLA rats. During anaesthesia, the RHA rats showed a greater fall of body temperature and bradycardia than did the RLA rats. Diethylether anaesthesia reduced locomotor activity in the RHA rats, but had no effect in the RLA rats. In neither RHA nor RLA rats did anaesthesia plus orbital puncture, versus anaesthesia alone, influence body temperature, heart rate rhythm, locomotor and eating activity. The lack of effect of orbital puncture occurred both in the short term (within 2 h) and long term (within 48 hours) and thus this study indicates that orbital puncture had, at least with respect to variables measured in the present study, no effect superimposed on that of diethylether anaesthesia.

Keywords Catecholamines; corticosterone; defaecation; diethylether anaesthesia; discomfort; glucose; micturition; orbital bleeding; telemetry

Orbital bleeding is a technique used frequently in rats (Angelov *et al.* 1984), but it is controversial, particularly due to arguments of an emotional nature (Van Herck *et al.* 1992a). In the Netherlands, diethylether is the anaesthetic of choice when collecting blood from the orbital venous plexus (Van Herck *et al.* 1992a). Assessment of behaviour of rats and their clinical condition after orbital bleeding showed no changes apart from a slightly higher incidence of enophthalmus in

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punctured eyes (Beynen *et al.* 1988a, Beynen *et al.* 1988b). Histopathological investigation of punctured orbits revealed haemorrhages in the puncture track and periosteum. Four days after bleeding, inflammatory reactions were present in the puncture track, eye muscles, periosteum and Harderian gland. Within four weeks after bleeding, the lesions had healed without detectable scars (Van Herck *et al.* 1992b, Krinke *et al.* 1988). Orbital bleeding in rats while they were under diethylether anaesthesia did not induce an endocrine stress response that differed from that caused by diethylether alone (Van Herck *et al.* 1991).

The abovementioned variables are considered sensitive indicators of physical and emotional stress in rats (Hendriksen & Koetter 1991, Manser 1992, Van Herck *et al.* 1994), but chronobiological alterations in behaviour and physiology (Koolhaas *et al.* 1990, Wiepkema & Koolhaas 1993) as induced by orbital puncture cannot be excluded. Thus, in this study we addressed the question of whether orbital bleeding in rats, while they are under diethylether anaesthesia, affects their diurnal locomotor activity, body temperature, heart rate and eating pattern. Orbital bleeding in anaesthetized rats was compared with both diethylether anaesthesia alone and sham anaesthesia. To enhance generalization of the results, we used two rat lines, differing in behavioural and physiological reactions to stressors, i.e. so-called RHA and RLA rats.

Materials and methods

The research project has been approved by the Ethics Committee of Veterinary Faculty, the experimental protocol by the Animal Ethics Committee of the Department of Laboratory Animal Science, Utrecht University.

Animals and housing

Male, conventional Roman High Avoidance (RHA/Verh) and Roman Low Avoidance (RLA/Verh) rats from the breeding colony of the Behavioural Biology Laboratory, Zurich, Switzerland, were kindly provided by Dr P. Driscoll. The rats had been used in a behavioural experiment (Meerlo *et al.* in press), in which some had a battery-operated radiotransmitter (TA11CTA-40, Data Sciences Inc., St Paul, USA) implanted intraperitoneally for registration of locomotor activity, body core temperature and heart rate, as described by Brockway *et al.* (1991). The other animals had received a type TA10TA-F40 transmitter for registration of locomotor activity and body core temperature. Implantation of the transmitter was done under diethylether anaesthesia when the rats weighed 275–300 g. All implanted materials were sterilized before use. From the day of surgery, the rats were housed individually in clear, perspex cages ($25 \times 25 \times 30$ cm) with a mesh-wire top and sawdust bedding (BMI, Helmond, The Netherlands). The cages were placed in a climatized room (temperature 20–20°C; relative humidity 55–65%) under a fixed day–night rhythm (light from 20:00 to 08:00 h). Tap water and commercial, pelleted rat feed (RMH-B®, Hope Farms BV, Woerden, The Netherlands) were provided *ad libitum*.

Experimental design

The experiment started six weeks after surgery, the eight RHA rats weighing 440 ± 12 g (mean $\pm$ SD) and the nine RLA rats weighing 417 ± 10 g. All animals were adapted to the environmental conditions and handling procedures. The experiment had a Latin-Square design with sham anaesthesia, diethylether anaesthesia and diethylether anaesthesia plus orbital bleeding as treatments. In order to assess the response to handling and novelty stress associated with anaesthesia, the rats were sham anaesthetized. Table 1 illustrates the type of measurements and treatment sequence in individual rats. Treatments were performed with an interval of one week.

According to the order given in Table 1, the animals were transported one by one, while in their home cage, to a room adjacent to the room in which they were housed. Before an animal was placed in the perspex anaesthesia box ($15 \times 15 \times 25$ cm), it was flushed for 2 min with either room air (sham anaesthesia) or with room air that had been led through a bottle containing diethylether mixed with

Table 1 Overview of type of measurements and treatment sequence in individual rats

Rat identification no. and treatment order	Rat strain	Telemetric measurements*	Treatment sequence†
16	RHA	A,T	A, A+OP, SA
13	RHA	A,T, E	A, A+OP, SA
10	RHA	A,T, H, E	A, A+OP, SA
7	RLA	A,T	A, A+OP, SA
4	RLA	A,T, E	A, A+OP, SA
1	RLA	A,T, H, E	A, A+OP, SA
17	RHA	A,T	A+OP, SA, A
14	RHA	A,T, E	A+OP, SA, A
11	RHA	A,T, H, E	A+OP, SA, A
8	RLA	A,T	A+OP, SA, A
5	RLA	A,T, E	A+OP, SA, A
2	RLA	A,T, H, E	A+OP, SA, A
15	RHA	A,T	SA, A, A+OP
12	RHA	A,T, H, E	SA, A, A+OP
9	RLA	A,T	SA, A, A+OP
6	RLA	A,T, E	SA, A, A+OP
3	RLA	A,T, H, E	SA, A, A+OP

*A=locomotor activity,T=body core temperature, H=heart rate, E=eating activity. A,T and H were measured as described by Brockway *et al.* (1991) and E as described by Strubbe *et al.* (1986)
†SA=sham anaesthesia, A=anaesthesia, A+OP=anaesthesia+orbital puncture

water. Between animals, the box was cleaned with a tissue containing chlorhexidine in 70% ethanol.

The animals were considered to be under diethylether anaesthesia as soon as the palpebral reflex had disappeared and were then removed from the perspex box. For orbital bleeding, an experienced animal technician penetrated the conjunctiva near the inner canthus of the right eye with an intact, heparinized Pasteur's pipette. The pipette was gently rotated as it was advanced through the conjunctiva alongside the eyeball into the vessels. As soon as blood appeared in the pipette, it was held still. One ml of blood was collected. After removal of the pipette, further bleeding was prevented by pressing cotton wool on the inner canthus of the punctured eye. Immediately after treatment, the animals were placed back in their cages and returned to their room.

Biotelemetric recordings

Except during the three treatments, each lasting about 7 min, locomotor activity, body temperature, heart rate and eating activity were recorded with intervals of 10 min. Recording was continuous until three days after the third treatment period. Heart rate, body core temperature and locomotor activity were measured using the hardware and software data acquisition system of Data Sciences Inc., consisting of a platform receiver (RA-1010) placed underneath the rat's cage to pick up the radio signals emitted from the implanted transmitters. The temperature-dependent biotelemetric signal was transferred via a consolidation matrix (BCM100, Data Sciences Inc.) located in the animal room, to a computer-based data acquisition system (Dataguest IV, version 2.21, Data Sciences Inc.) located remotely in a separate room. The receiver detects activity by recording changes in position of the implanted biotelemetric device. A change in the horizontal position of the transmitter alters the strength of the signal that is detected by the receiver. Every change in the signal strength is recorded as an activity event. The computer (486/66) was configured to record in cyclic runs of 10 min. During each cycle, each rat was measured for 10 s with a sampling rate of 500 Hz for ECG recording and 250 Hz for body core temperature recording. Mean values of heart rate,

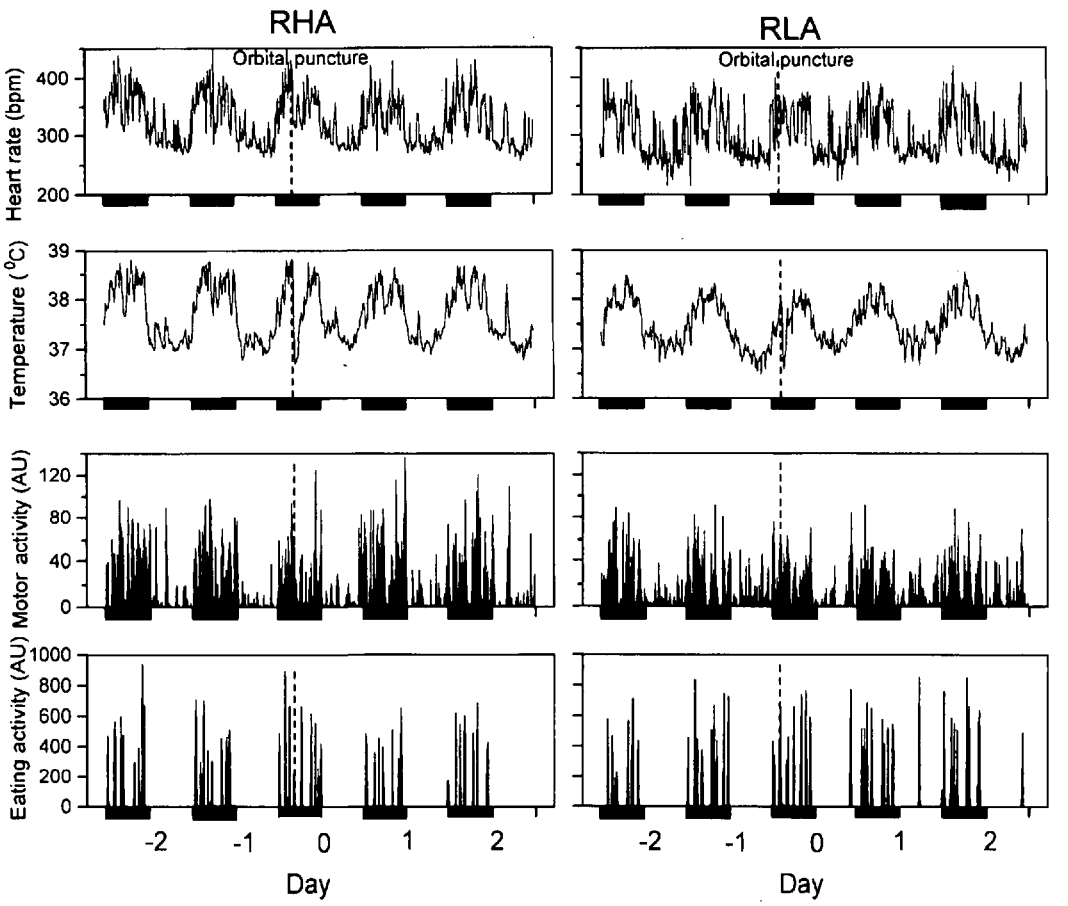

Fig 1 Five-days diurnal patterns of heart rate, body temperature, locomotor and eating activity in a typical RHA and a RLA rat. On day 0, at 11:00 h, orbital bleeding was performed as indicated by the broken vertical line. Solid segments on the X-axis indicate the 12-h dark periods

body core temperature and locomotor activity were then computed and stored. Heart rate is expressed in beats per minute, body core temperature in °C, locomotor activity in number of movements (units) per 10 min and eating activity as number of movements within the foodhopper (units) per 10 min. Means of values recorded over a 12 h light and 12 h dark period were calculated using Dataquest analysis routines.

Miscellaneous measurements

During treatments, the following variables were scored if appropriate: time needed to induce anaesthesia (palpebral reflex absent), time needed to perform orbital bleeding and time needed for recovery (righting reflex present). While in the anaesthesia box, animals were observed for signs of excitation (yes/no), defaecation (yes/no) and micturition (yes/no). In blood samples, plasma adrenaline, noradrenaline, corticosterone and glucose were determined as described previously (Van Herck *et al.* 1991). To obtain undisturbed, basal values for the plasma variables, blood samples were also taken from a separate group of RLA (n=7) and RHA (n=7) rats provided with a chronically implanted jugular vein catheter.

Statistical analyses

Results for continuous parameters (Fig 2, Tables 2 and 4) are presented as means ± SD. The Kolmogorov–Smirnov one-sample test was used to check normality of the continuous data. All results within the groups were

Table 2 Induction and recovery times for diethylether anaesthesia in RHA and RLA rats

Strain	Induction (s)		Recovery (s)	
	A*	A+OP*	A	A+OP
RHA (n=8)	85±18	89±21	77±17	87±25
RLA (n=9)	104±21	97±25	56±19	43±12

Results expressed as means±SD. Recovery, but not induction times differed significantly between strains ($P<0.001$, MANOVA, repeated measurements), whereas recovery times did not differ between treatments. *A=anaesthesia, A+OP=anaesthesia+orbital puncture

found to be normally distributed. Telemetric data were subjected to a multivariate analysis of variance (MANOVA, repeated measurements) with strain as between-subject factor, and treatment and time as within-subject factors. Times needed for anaesthesia or recovery were analysed with (MANOVA, repeated measurements) strain as between-subject factor and treatment as within-subject factor.

The scores from excitation, defaecation and micturition were pooled for the two diethylether treatments and the Fisher's exact test was used to identify strain differences. Plasma variables were subjected to the unpaired Student's *t*-test to identify strain and bleeding method differences. In case of the analyses of variance (MANOVA, repeated measurements), the homogeneity of variances was checked using Bartlett's test. The variances were similar. In all cases the probability of a type I error <0.05 was taken as the criterion of significance. Two side probabilities were estimated throughout. All statistical analyses were carried out according to Steel and Torrie (1981) using a SPSS PC+ computer program (Release 4.0; SPSS Inc., Chicago, USA).

Results

Biotelemetric variables

Under undisturbed conditions, RLA and RHA rats showed circadian patterns of heart rate, body core temperature, locomotor activity and eating activity, with high values during the dark, and low values during the light phase (Fig 1). The patterns did not differ between the two strains (not shown). After sham anaesthesia, the RHA and RLA rats displayed a similar, transient increase in heart rate and locomotor activity (Fig 2). After diethylether anaesthesia, both strains reacted with a transient bradycardia and a pronounced hypothermia which lasted about 2 h. Orbital puncture had no additional influence. The hypothermic and bradycardiac responses were greater ($P<0.05$, MANOVA) in RHA than RLA rats. Anaesthesia plus orbital puncture versus diethylether anaesthesia alone did not affect locomotor activity. In RLA rats, locomotor activity increased after anaesthesia with or without orbital puncture, whereas RHA rats showed no increase (Fig 2). Neither diethylether anaesthesia alone nor anaesthesia plus orbital puncture influenced the circadian patterns of the telemetric variables 5 h after treatment, when compared with sham anaesthesia (not shown). Eating activity was not influenced by any of the three treatments.

Miscellaneous measurements

The time needed to induce anaesthesia did not differ between strains, but the recovery time was significantly shorter in RLA than RHA rats (Table 2). During sham anaesthesia no excitation was observed whereas diethylether anaesthesia caused excitation in about one-third of the animals (Table 3). The incidence of defaecation was not influenced by diethylether anaesthesia. During induction of the diethylether anaesthesia RLA rats urinated significantly less than RHA rats. On average, it took 17±3 s (mean±SD, $n=17$) to perform orbital bleeding.

While the rats were under diethylether anaesthesia, the plasma concentrations of adrenaline, noradrenaline and glucose were significantly higher in RHA than RLA rats (Table 4). Basal levels of these variables, as obtained from awake animals with a jugular

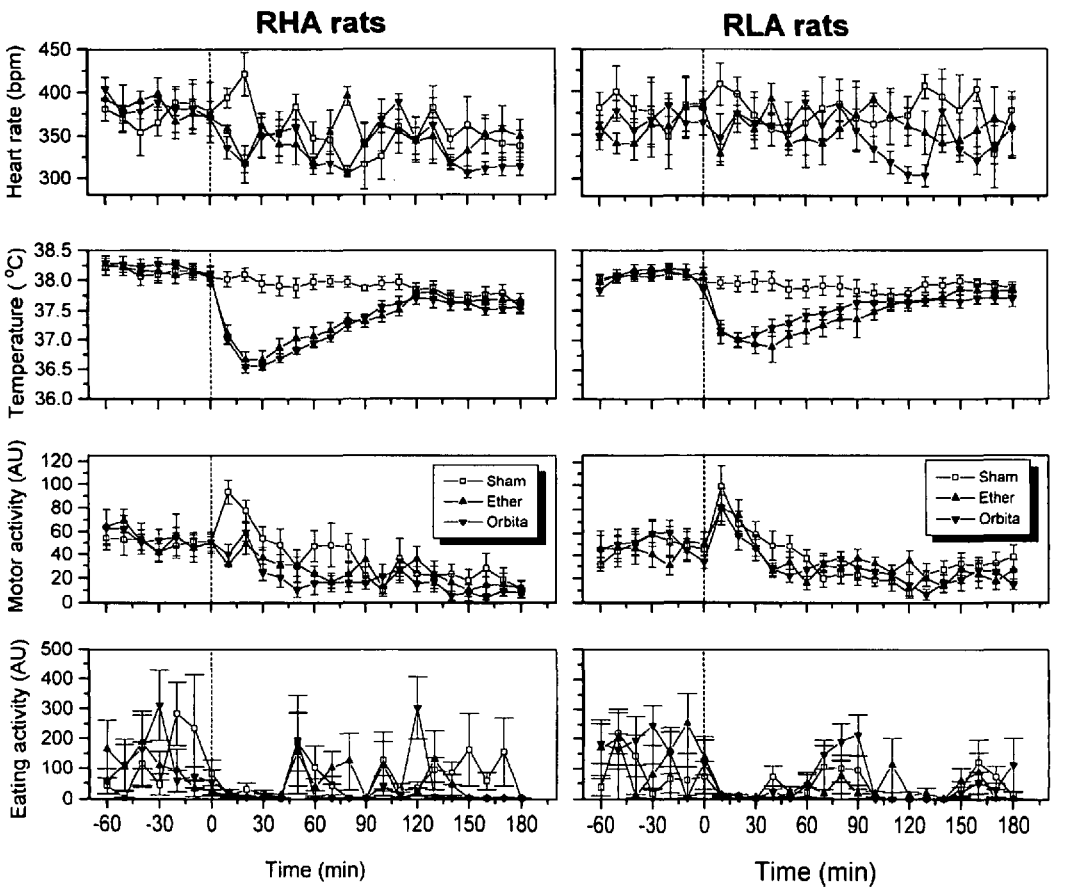

Fig 2 Short-term effects of sham anaesthesia and diethylether anaesthesia with or without orbital bleeding on body temperature, heart rate, and locomotor activity in RHA and RLA rats. The zero time point corresponds to the onset of treatment. Results are presented as means ± SD (vertical bars) for eight RHA and nine RLA rats

vein catheter, were similar for the two strains. Except for corticosterone, the basal levels were markedly lower than those seen after anaesthesia plus orbital puncture.

Discussion

The short and long-term impact of diethylether anaesthesia plus orbital puncture on telemetrical variables was studied in RHA and RLA rats. The two strains are derived from Wistar rats, differing in the degree of their active avoidance behaviour in a shuttle box with light as conditioned and electric shock as unconditioned stimulus (Driscoll & Battig 1982). It was reasoned that the use of rats with different behavioural responses would widen the basis for generalization of

Table 3 Frequency of excitation, defaecation and micturition when the rats were in the anaesthesia box

	Excitation		Defaecation		Micturition	
Treatment	RHA	RLA	RHA	RLA	RHA	RLA
Sham	0/8	0/9	4/8	5/9	2/8	2/9
Anaesthesia	5/16	8/18	9/16	11/18	6/16*	1/18

Results expressed as number of positive rats/total number of rats; for the treatments anaesthesia alone and anaesthesia plus orbital puncture the data were combined. *Significantly different from the RLA rats ($P < 0.05$, Fisher's exact test)

Table 4 Plasma corticosterone, adrenaline, noradrenaline and glucose levels in the blood samples derived from RHA and RLA rats by either orbital puncture or a jugular vein catheter

Plasma variable	Orbital bleeding		Jugular catheter	
	RHA (n=8)	RLA (n=9)	RHA (n=7)	RLA (n=7)
Adrenaline (pg/ml)	638*±112	320±125	75±58†	61±32‡
Noradrenaline (pg/ml)	1054*±125	588±80	190±48†	176±38‡
Corticosterone (μg/dl)	7.7±1.9	9.9±2.8	4.7±4.9	5.3±5.5
Glucose (mg/dl)	179*±11	138±5	106±8†	104±7‡

Results expressed as means±SD
*Significantly different from punctured RLA rats ($P<0.05$, Student's t-test)
†Significantly different from punctured RHA rats ($P<0.05$, Student's t-test)
‡Significantly different from punctured RLA rats ($P<0.05$, Student's t-test)

the outcome of this study. The two strains were indeed found to differ in their response to diethylether anaesthesia. The RHA rats needed more time to recover from the anaesthesia, urinated more during anaesthesia and showed a greater endocrine stress response to anaesthesia when compared with the RLA rats. Thus, the RHA rats can be considered more sensitive to stress caused by diethylether inhalation than the RLA rats.

The hyper-sensitivity to diethylether in the RHA rats is also mirrored in the telemetrical measurements. The RHA rats showed a greater fall in body temperature and greater bradycardia than did the RLA rats. Diethylether instead of sham anaesthesia reduced locomotor activity in the RHA rats, but had no effect in the RLA rats.

It is clear that the telemetrical measurements were significantly affected by the diethylether versus sham anaesthesia, the magnitude of the effect being dependent on the strain of rats studied. However, in neither RHA nor RLA rats did anaesthesia plus orbital puncture, versus anaesthesia alone, influence body temperature, heart rate rhythm, locomotor and eating activity. There was neither a short-term (within 2 h) nor a long-term (within 48 h) effect of orbital puncture. Thus, it can be concluded that orbital puncture had no effect superimposed on that of diethylether.

This study shows that diethylether anaesthesia had pronounced effects on body temperature, heart rate, and locomotor activity. Any effect of orbital puncture per se might have been overruled by the marked effect of diethylether anaesthesia. The present results confirm an earlier study, showing that orbital puncture in rats while under diethylether anaesthesia did not produce an additional endocrine stress response (Van Herck *et al.* 1991).

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